Supercooling Points of *Lysiphlebus testaceipes* and Its Host *Schizaphis graminum*

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ABSTRACT Supercooling points (SCPs) were measured for various life stages of male and female *Lysiphlebus testaceipes* (Cresson) parasitoids, along with mummies and its aphid host, *Schizaphis graminum* (Rondani). Some parasitoids were acclimated (4 h at 10°C before cooling down to the SCP) to determine whether this could significantly lower the SCP. Acclimation did not improve SCPs for *L. testaceipes.* An inverse relationship between age of the adult parasitoid and its SCP was detected. Nonacclimated male and female parasitoids older than 12 h after emergence spontaneously froze at the warmest mean temperatures (-20.32 ± 1.32 and -22.55 ± 0.62°C [SE], respectively). Younger female adult parasitoids (<6 h after emergence) and mummies had mean SCPs less than -26°C. The SCP for the greenbug host was slightly warmer at -25.98 ± 0.10°C. Knowledge of SCPs for *L. testaceipes* and its host *S. graminum* help provide insights about their ability to successfully function throughout the winter in the southern Great Plains.

KEY WORDS *Lysiphlebus testaceipes*, *Schizaphis graminum*, supercooling point, winter hardiness

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Aphidiidae) is a nearctic parasitoid that attacks >100 aphid species (Mackauer and Starý 1967, Starý et al.1988, Pike et al.2000). It is an important parasitoid of cereal aphids in the southern Great Plains and has been observed to suppress winter wheat (*Triticum Aestivum* L.) pests such as the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae), below economic injury levels (EILs) (Hight et al. 1972, Jones 2001, Giles et al. 2003).

Climatic conditions in Oklahoma are relative mild during the winter months of wheat growth (Table 1). In January, low temperatures average -3°C and highs average 8°C in Oklahoma City from 1973 to 2003 (NOAA). This transitional area of the Great Plains, however, occasionally experiences dramatic drops in temperature, with extremes reaching as low as -22°C (Table 1). When temperatures approach colder extremes in the southern Great Plains, little is known about the relationship between *L. testaceipes* and its greenbug host. Predictability of *L. testaceipes* for helping regulate greenbug populations is dependent on its survival during periods of potentially fatal cold winter weather.

Insects exposed to temperatures below the melting point of their body fluid are in danger of being killed by a lethal freezing of that fluid (Salt 1961, Baust 1973, Block 1995). In general, one of two strategies enables insects to survive such extreme conditions. They may be able to tolerate being in a frozen state, or they avoid freezing by various methods including supercooling their body fluids to a point below their actual melting point (Zachariassen 1985). The supercooling point (SCP) of an insect can be lowered by a number of means, including production of gylcerol and other antifreeze compounds, dehydration of the insect, ingestion of certain substances, or changes in fatty acids (Sømme 1982). SCPs provide a basic indication as to what the coldest temperature extreme is that an insect could survive (Salt 1961, Leather et al. 1993).

The objectives of this study were to (1) determine the supercooling points of various life stages *of L. testaceipes* and its greenbug host, and (2) determine whether conditioning at an intermediate temperature would significantly alter the SCP. Knowledge of these SCPs would indicate the coldest possible temperature extremes that *L. testaceipes* could survive and provide insights about population interactions with greenbugs during the winter.

Materials and Methods

Insect Preparation. Wheat seed (cultivar 2137) was planted in 5-cm-diameter by 20-cm-tall Ray Leach "conetainers" (Stuewe & Sons, Corvallis, OR) and grown for ≈3 wk. A clear acetate tube cage (5 cm diameter by 30 cm tall) was fitted around the top of the conetainer as previously described by Jones et al. (2003). To provide adequate ventilation, each cage had two holes in the sides covered with polyester fine mesh netting.

Conetainers of wheat were infested with 25–50 third-instar and older greenbugs from a previously

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Table 1. Temperatures for Oklahoma City, OK (1973–2003), from the National Virtual Data System, part of the NOAA (http://nndc.noaa.gov/)

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April
High (normal)	29°C	23°C	16°C	10°C	8°C	12°C	17°C	22°C
Low (normal)	17°C	11°C	3°C	−2°C	−3°C	−1°C	4°C	9°C
Maximum	42°C	36°C	31°C	30°C	27°C	33°C	34°C	38°C
Minimum	2°C	−9°C	−12°C	−22°C	−20°C	−19°C	−16°C	−7°C

described stock colony of biotype E greenbugs (Jones et al. 2005). These greenbugs were allowed to settle overnight, after which five male/female pairs of *L. testaceipes* from parasitoid colonies reared on wheat (Jones et al. 2005) were released into each conetainer cage. By limiting the number of greenbugs, the fitness of emerging parasitoids was not influenced by plant health (Fuentes-Granados et al. 2001). Parasitized greenbugs were allowed to develop into mummies, after which they were removed daily from the conetainer and placed individually into 1.5-ml microcentrifuge vials. Vials were kept at 22 ± 1°C and a photoperiod of 12:12 (L:D). Mummies were allocated for mummy SCP analysis or allowed to develop to provide adult *L. testaceipes* for adult SCP analysis. Greenbugs destined for SCP analysis were treated similarly, with adult greenbugs being introduced into conetainers of wheat and reared for 24 h at 22 ± 1°C and a photoperiod of 12:12 (L:D).

Measurement of SCP. To measure the SCP, four thermocouple chambers were constructed (Fig. 1). Each consisted of a 2.5 by 20-cm test tube, with a cork stopper. A 0.3 by 10-cm wooden dowel was placed in the center of the stopper so that it would hang down into the center of the test tube and provide a platform to place the insect specimen on. A 1-m-long copper-constantan standard gauge thermocouple wire was extended through the cork stopper and taped to the support dowel. To the distal end of the standard gauge wire, a short length of ultra-fine copper wire was soldered to the end of the copper wire, and the same procedure was performed to the constantan lead using constantan ultra-fine wire. The distal ends of the ultra-fine wires were twisted together and secured with a small drop of solder to create the temperature-measuring interface.

Each insect specimen (greenbug, *L. testaceipes* mummy, or *L. testaceipes* adult) was placed singly onto the end of the wooden dowel along with a small drop of petroleum jelly to secure the insect. The ultra-fine thermocouple was placed in contact with the insect specimen and secured with more petroleum jelly. The specimen was centered into the test tube. Each thermocouple was connected to a Sable Systems International TC-1000 thermocouple meter (Sable Systems International, Henderson, NV). The TC-1000 meter self-calibrated (<0.2°C) against laboratory-grade internal standards when turned on and at regular intervals while temperature measurements were occurring. Temperature measurements were relayed from the meter to a laptop computer through a serial cable and were recorded to an ASCII text file. Once connected, each test tube was placed, along with three other test tube–thermocouple preparations, inside a wrapping of foam insulation (≈5 cm thick). This is done to slow the cooling rate to ≈1°C/min. This group of insulated test tubes was placed into a styrofoam box and surrounded with crushed dry ice. Temperature measurements were taken every 0.5 s until the exotherm associated with the latent heat of crystallization is detected (Fig. 2). The onset of the exotherm corresponds with the SCP for each specimen (Salt 1961).

As insects were available, we determined mean SCPs for the greenbug host, *L. testaceipes* mummies
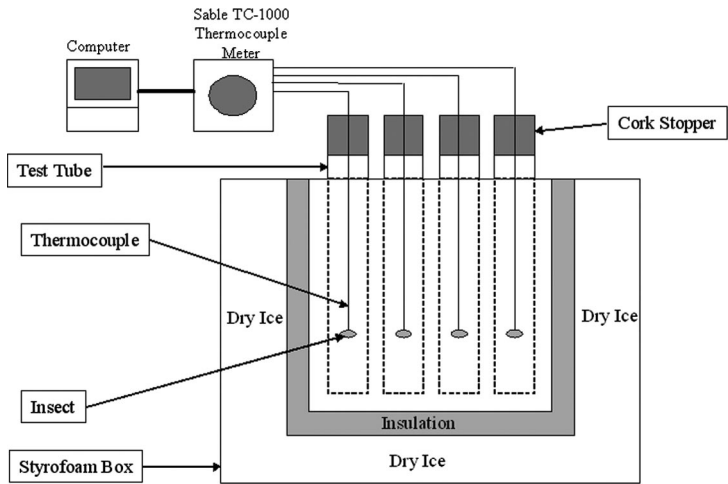

Fig. 1. Generalized diagram of supercooling point measuring equipment as used in this experiment.
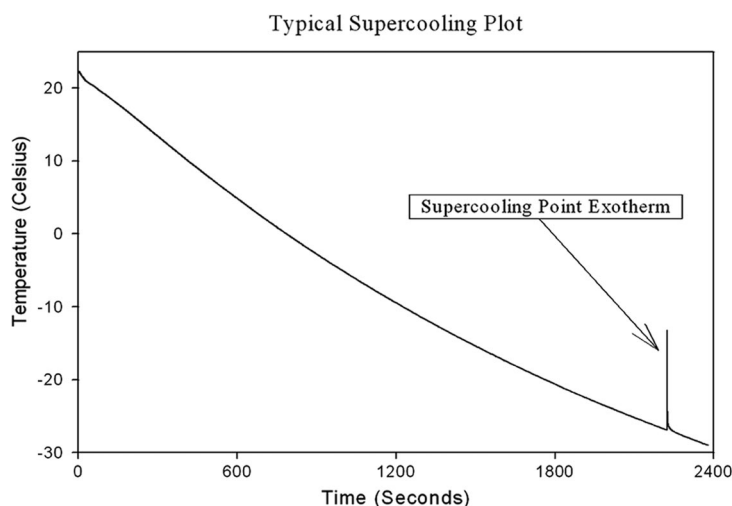
Fig. 2. Generalized plot showing the exotherm associated with the latent heat of crystallization. The onset of the exotherm corresponds with the supercooling point for each specimen (Salt 1961).

(mummies consisted of greenbugs that had mummified within the previous 24 h), freshly emerged adult parasitoids (<6-h postemergence males and females), and older parasitoids (>12-h postemergence males and females). Minimums of 20 individual specimens were used to determine each mean SCP.

Acclimation at an intermediate temperature for a few hours can improve the SCP because of changes in the insect's physiology (Sømme 1982, Lee 1991). Most aphid parasitoids including *L. testaceipes* are polyvoltine, as are their hosts including the greenbug and as such usually respond to daylength and temperature as cues for the induction of diapause and diapause-associated changes such as increased cold hardiness (Polgár and Hardee 2000). To determine whether acclimation significantly changed the SCP of *L. testaceipes* males, females, and mummies, these life stages were acclimated at an intermediate temperature of 10°C for 4 h before measuring those SCPs. Acclimation at 10°C was chosen because it represents the approximate average daily temperature during November, before the onset of the coldest trmperatures during December and January (Table 1). Because *L. testaceipes* is a

rather short-lived parasitoid (unpublished data), acclimation time was kept short at 4 h. These acclimated adult parasitoids were categorized as being 6- to 10-h postemergence specimens.

Statistical Analysis. An analysis of variance (ANOVA) was performed using PROC MIXED in PC SAS version 8.2 (SAS Institute 1999) to compare SCPs among specimens at a significance level of $P = 0.05$. Student's t-test was used to compare differences in mean SCP across treatments.

Voucher Specimens. Voucher specimens of *L. testaceipes* adults, mummies, and *S. graminum* adults were deposited in the Department of Entomology and Plant Pathology museum at Oklahoma State University in Stillwater.

Results and Discussion

Mean SCPs for all treatments ranged from −20.32°C for older male parasitoids to −26.33°C for mummies acclimated at 10°C (Table 2). There were significant differences across treatments ($F_{8,218} = 8.72$, $P < 0.0001$). Acclimated and nonacclimated mummies,

Table 2. SCPs for greenbug, *L. testaceipes* mummies, freshly emerged adults, older adults, and adults conditioned at 10°C

Species	Acclimation	Life stage	Sex	Mean SCP ± SE (°C)	n	SCP range (°C) (max, min)	Sig[a]
L. testaceipes	4 h @10°C	Mummy	NA	−26.33 ± 0.20	19	−24.49, −27.79	A
L. testaceipes	None	Adult (<6 h after emergence)	Female	−26.13 ± 0.31	22	−22.25, −27.48	AB
Greenbug	None	Adult	Female	−25.98 ± 0.10	22	−25.03, −26.76	ABC
L. testaceipes	None	Mummy	NA	−25.94 ± 0.18	20	−24.49, −27.16	ABC
L. testaceipes	None	Adult (<6 h after emergence)	Male	−24.40 ± 0.45	27	−19.50, −26.97	BCD
L. testaceipes	4 h @10°C[b]	Adult (<6 h after emergence)	Male	−23.95 ± 0.50	20	−19.30, −26.49	CDE
L. testaceipes	4 h @10°C[b]	Adult (<6 h after emergence)	Female	−23.29 ± 0.61	20	−19.23, −26.53	DE
L. testaceipes	None	Adult (>12 h after emergence)	Female	−22.55 ± 0.62	48	−9.29, −26.74	E
L. testaceipes	None	Adult (>12 h after emergence)	Male	−20.32 ± 1.32	29	−5.40, −27.26	F

[a] Treatments followed by the same letter had mean SCPs that were not significantly different ($P \leq 0.05$).
[b] Adult specimens that were acclimated for 4 h @ 10° C are described in the text as being 6–10 h after emergence.

Table 3. Coldest SCPs for adults of various aphid and parasitoid species

Species	SCP (°C)	Reference
Lysiphlebus testaceipes Cresson	−26.13	This paper
Aphidius colemani Viereck	−25.4	Hofsvang and Hägvar 1977
Eretomocerus eremicus (Rose & Zolnerowich)	−25.0	Tullett et al. 2004
Ephedrus cerasicola Starý	−26.1	Hofsvang and Hägvar 1977
Schizaphis graminum (Rondani)	−25.98	This paper
Diurahpis noxia Mordiilko	−24.9	Butts 1992
Aphis glycenes Matsumura	−24.9	McCornack et al. 2005
Megoura crassicauda Mordvilko	−24.5	Asai et al. 2002
Myzus persicae (Sulzer)	−24.2	Bale et al. 1988
Sitobion avenae (F.)	−24.2	Knight et al. 1986
Acyrthosiphon pisum (Harris)	−23.7	Asai et al. 2002
Aphis fabae Scopoli	−23.6	O'Doherty 1986
Elatobium abietinum (Walker)	−15.7	Powell 1974

greenbugs, and nonacclimated female *L. testaceipes* adults had the lowest mean SCPs but were not significantly different from one another (df = 218, t < 1.82, P > 0.07). This lack of significant difference in SCPs was not unexpected because parasitoid mummies and their hosts are closely related with respect to their body resources (Brodeur and Boivin 2004). These SCPs for greenbug and *L. testaceipes* are similar to similar species including other cereal aphids such as English grain aphid, *Sitobion avenae* (F.), aphid parasitoids *Aphidius colemani* Viereck, and *Ephedrus cerasicola* Starý and the whitefly parasitoid *Eretmocerus eremicus* (Rose and Zolnerowich) (Table 3).

A general trend was discerned that, as the parasitoid ages, its ability to supercool is reduced (Fig. 3). SCPs for older parasitoids were significantly higher than for all other treatments (Table 2). Nonacclimated older male parasitoids (older than 12 h after emergence) spontaneously froze at the warmest mean temperature (−20.32 ± 1.32°C). Nonacclimated older female parasitoids (>12 h after emergence) had a significantly

lower mean SCP of −22.55°C (df = 218, t = 2.68, P = 0.008). Additionally these two groups had at least a 3-fold larger range compared with any of the other groups (Table 2).

For many insects, acclimation for a short period of time at an intermediate temperature can significantly lower the SCP (Sømme 1982, Lee 1991). We determined that acclimation for 4 h at 10°C had no significant effect on mean SCP for *L. testaceipes* mummies (−26.33 versus −25.94°C; df = 218, t = 0.34, P = 0.73). Additionally, there were no significant differences in mean SCP for acclimated and nonacclimated male *L. testaceipes* (−24.40 versus −23.95°C; df = 218, t = 0.43, P = 0.66). However, the mean SCP for acclimated female *L. testaceipes* was significantly warmer than for nonacclimated females (−26.13 versus −23.29°C; df = 218, t = 2.60, P = 0.01).

The observation that acclimation did not significantly lower the SCP for *L. testaceipes* (Table 1) indicated that either (1) no changes were taking place in the parasitoids that could enable them to withstand lower temperatures (Sømme 1982) or (2) our acclimation "treatment" of 10°C for 4 h was insufficient to initiate such changes to occur. Additionally, the photoperiod was not changed in this experiment, and perhaps *L. testaceipes* needs an additional daylength cue to alter its SCP. Acclimated adult parasitoids had SCPs that were intermediate to the nonacclimated adults (<6 h after emergence) and the older parasitoids (>12 h after emergence). These acclimated adult parasitoids were on average ≈4 h older than the freshly emerged parasitoids and at least 2 h younger than the older parasitoids. If *L. testaceipes* is indeed able to lower its SCP, other factors may be important, such as the physiological state of its host or perhaps even the species of the host aphid (Polgár and Hardee 2000). Progeny of *Aphidius matricariae* Haliday would not enter diapause if anholocyclic aphid species such as *Aphis gossypii* Glover and *Myzus ascalonicus* Doncaster were the host aphid. However, *A. matricariae*
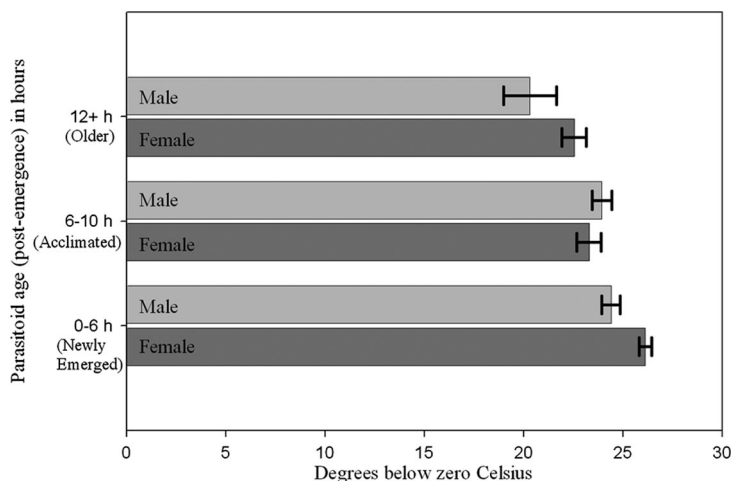
Fig. 3. Plot of supercooling points of *L. testaceipes* as related to adult parasitoid age (hours postemergence from mummy).

could enter diapause if they developed in apterous virginoparae of the holocyclic aphid species *Myzus persicae* Sulzer (Polgár and Hardee 2000).

Supercooling ability can often be attributed to the accumulation of cryoprotectant chemicals and/or the absence on ice nucleating agents (Lee 1991). Sugars such as glucose, trehalose, and fructose and polyols such as glycerol, mannitol, and sorbitol are known to provide increased supercooling ability and are commonly found in insects (Tanno 1964, Sømme 1967, 1969, Block and Zettel 1980). Perhaps, common sugars, such as trehalose, constitute a high percentage of *L. testaceipes* hemolymph and provide much of their supercooling ability. Depletion of this sugar or some other resource necessary for the *L. testaceipes* parasitoid to live may be responsible for the inverse relationship between the SCP and the age of the parasitoid. Another possibility was that, as the parasitoids aged, they accumulated ice-nucleating agents in their hemolymph as a by-product of normal metabolic processes, allowing individuals to freeze at warmer temperatures.

In Oklahoma, parasitoids experience temperatures that range from ideal, to tolerable, to unsuitable, during the winter wheat growing season (Table 1). Based on SCP, *L. testaceipes* could survive even the most extreme temperatures experienced in central Oklahoma (Table 1). However SCP is only a theoretical base value of how cold an organism can be before it freezes (Bale 1993). The parasitoid may perish or be rendered unable to function in a normal manner at much warmer temperatures than its SCP. This is shown by studies of the English grain aphid, a potential host of *L. testaceipes*, which perishes at temperatures below −14.6°C, ≈12°C above its SCP (Parish and Bale 1991).

Lysiphlebus testaceipes is commonly found at latitudes that experience much colder temperatures than Oklahoma (Royer et al. 2001). How these parasitoids survive the winter at the colder latitudes is yet unknown. Do these parasitoids survive the winter or do they immigrate in from the south each spring? If *L. testaceipes* is able to overwinter in these colder climates, it may be that they need protection by snowfall much in the same manner that snowfall insulates and protects overwintering Colorado potato beetles (Milner et al. 1992, Hoy 1998). Another possible method of winter survival could be that *L. testaceipes* mummies are able to survive temperatures closer to their SCP than their other life stages can. More research is needed to determine more precisely the temperatures and winter conditions *L. testaceipes* can survive in Oklahoma.

Acknowledgments

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